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DEVELOPMENT OF A THERAPEUTIC AGENT FOR WOUND-HEALING ENHANCEMENT

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SCIENTIFIC PROGRESS DURING THIS QUARTER

Peptide Synthesis

During this quarter we prepared four peptides, as listed below:

PDGF A (53-63)	Peptide #5, Table 3, p. 18 in the original proposal
PDGF A (66-78)	Peptide #6, Table 3, p. 18 in the original proposal
PDGF A (77-88)	Peptide #7, Table 3, p. 18 in the original proposal
PDGF B (25-42)	Peptide #2, Table 4, p. 18 in the original proposal

Biological Assays

During this quarter we conducted cell-binding experiments and assays for mitogenesis and chemotaxis on the four peptides mentioned above. The results are shown in Tables 1 through 3. None of these peptides showed any cell binding or mitogenesis. PDGF A (77-88) showed chemotaxis. We are repeating the assay for this peptide.

Table 1
RESULTS OF COMPETITIVE BINDING ASSAY

Peptide	Concentration (ng or μg per well)	CPM	Average ± SD	Maximum Binding	Inhibition (%)
^{125}I PDGF	5 ng	37,890 33,988 38,143	36,674 ± 2,329	100	0
PDGF BB	50 ng	19,689 21,135 18,053	19,626 ± 1,542	54	46
	25 ng	22,427 22,049 23,350	22,609 ± 669	62	38
	12.5 ng	29,197 29,662 29,717	29,525 ± 286	81	19
	6.25 ng	32,486 34,744 34,903	34,044 ± 1,352	93	7

Table 1 (concluded)

	3.125 ng	35,150 37,819 37,923	36,964 ± 1,572	100	0
	1.56 ng	36,002 37,475 36,137	36,538 ± 814	100	0
PDGF A (53-63)	500 µg	31,288 31,691 32,193	31,724 ± 453	87	13
	250 µg	36,764 37,412 27,440	33,872 ± 5,580	83	17
	125 µg	37,063 37,758 38,405	37,742 ± 671	92	8
PDGF A (66-78)	500 µg	39,403 37,505 37,546	38,151 ± 1,084	93	7
	250 µg	39,071 41,365 39,426	39,954 ± 1,235	98	2
	125 µg	40,465 40,564	35,349 ±	91	9
PDGF A (77-88)	500 µg	40,071 37,691 38,879	38,880 ± 1,190	100	0
	250 µg	38,983 38,805 39,532	39,107 ± 379	100	0
	125 µg	38,631 38,176 37,584	38,131 ± 523	98	2
PDGF B (25-42)	500 µg	32,668 32,415 33,447	32,843 ± 538	80	20
	250 µg	29,453 31,978 31,483	30,972 ± 1,338	76	24
	125 µg	35,117 34,305 36,278	35,233 ± 992	86	14

Table 2
THYMIDINE INCORPORATION AT PEPTIDE CONCENTRATION (μg/ml)

Peptide	Control (media)	1000	500	250
PDGF A (53-63)	1,607 ± 305	1,137 ± 73	1,427 ± 197	1,761 ± 118
PDGF A (66-78)	1,544 ± 174	1,613 ± 121	1,759 ± 208	1,532 ± 276
PDGF A (77-88)	1,474 ± 315	1,507 ± 143	1,630 ± 297	1,637 ± 147
PDGF B (25-42)	1,961 ± 144	2,664 ± 943	1,828 ± 218	1,902 ± 209
PDGF	100 ng	11,681 ± 911		
	10 ng	3,935 ± 336		
	1 ng	1,897 ± 271		
	Media	1,527 ± 282		

Table 3
CHEMOTACTIC ACTIVITY OF PEPTIDES

Peptide	Concentration (mg or ng/ml)	OD_{650}[a]	SD	Chemotactic Activity
Media		0.015	0.002	—
PDGF A (53-63)	250 μg	0.011	0.002	—
PDGF A (66-78)	250 μg	0.023	0.025	—
PDGF A (77-88)	250 μg	0.053	0.010	+
PDGF B (25-42)	250 μg	0.006	0.006	—
PDGF	20 ng	0.030	0.014	+

[a]Peptides were considered positive when the OD was 1-1/2 times more than that of the media.

PLANS FOR NEXT QUARTER

During the next quarter we plan to continue the synthesis of peptides and to conduct cell-binding, mitogenesis, and chemotaxis assays.